unfolded state was formed which is in equilibrium with an aggregated, soluble state at pH 6 to 8. UV-CD data indicate that the secondary structure remains mostly intact. The heme is hexacoordinated low spin (pH > 6), likely with two axial histidines (His18, and His33). Below pH 6, a new ligation state emerges, for which spectroscopic evidence suggests a pentacoordinated quantum mixed state of the heme iron, previously found only in ferricytochrome c' and in class 3 peroxidases. Our data indicates the population of a frustrated misfolded state, occupied as a result of an alternate folding pathway, along which the glass transition precedes folding. Gel electrophoresis revealed that the protein is predominantly monomeric at low concentration (0.05 mM), while significant amounts of soluble dimers and trimers are formed at higher concentration, where we also observed a substantial fraction of reduced cytochrome c, which we assigned to the monomeric species based on results from size exclusion chromatography. We speculate that cytochrome c might adopt a similar state on the surface of liposomes and on the inner membrane of mitochondria have been found to acquire peroxidase activity, for which this state may be a prerequisite.

2889-Pos Board B44
Cytochrome *C* Binding to Liposomal Surfaces
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Cytochrome c is involved in triggering apoptosis in the mitochondria. In order to understand this initiation process of apoptosis, the interactions between cytochrome c and anionic lipid surfaces must be thoroughly characterized. It is well known that cytochrome c adopts partially unfolded conformations on cardiolipin containing liposomes. We examined the binding of cytochrome c to liposomes with different cardiolipin content by measuring the W59 fluorescence as a function of liposome concentration. Surprisingly, the thus obtained binding isotherms indicate a biphasic binding process, which are likely to correspond to different cardiolipin binding sites. Liposome concentrations associated with these binding sites were further investigated by far UV and visible CD as well as by absorption spectroscopy.The initial data obtained indicates that upon binding to cardiolipin complexes, cytochrome *c* adopts a nonnative state. This conformation is a low spin hexacoordinated state where the heme is ligated to H33 and H18. The far UV CD spectra shows that the protein retains its α-helical content regardless of CL binding, which is characteristic of native cytochrome *c*. The W59 signal in the near UV CD spectra disappears, indicating a more open protein conformation. This supports our fluorescence data which show an increase in fluorescence intensity as the W59 moves farther away from the heme. Soret band CD simulations also indicate an intermediate state upon protein binding to CL complexes. Data obtained from ultracentrifugation and gel electrophoresis experiments indicate a nonreversible mode of cytochrome *c* and liposome interactions, which might be due to the insertion of the lipids' hydrophobic tail into the protein crevice.

2890-Pos Board B45
Cytoglobin Interactions with Hydrophobic Probe 1,8-ANS
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Cytoglobin (Cygb) is small heme protein that belongs to the hexa-coordinate hemoglobin family. Cygb is over-expressed in fibrosis and neurodegenerative disorders, whereas it is down-regulated in some types of cancer including head and neck cancer. A recent report indicates that Cygb binds sodium oleate, which promotes conformational transition from hexa to penta-coordinated heme iron suggesting a possible role of Cygb in lipid signaling. To understand the mechanism of Cygb-lipids interactions, we have characterized the interactions between the hydrophobic probe 1-anilino-8-naphthalene sulfonate (1,8-ANS) and Cygb in the presence and absence of sodium oleate using steady-state and time-resolved fluorescence spectroscopy and isothermal titration calorimetry. Cygb binds 1,8-ANS in the ferric, ferrous and exogenous ligand bound form. Addition of sodium oleate to Cygb-ANS complex leads to a decrease in the 1,8-ANS emission intensity indicating competition between the lipid and ANS for Cygb binding sites. Two binding sites were identified using ITC for ANS binding to Cygb, one with moderate affinity (Kd ~ 50 μM) and a low affinity binding site (Kd ~ 2 mM). Reduction of the internal disulfide bond in Cygb slightly decreases the affinity of 1,8-ANS to Cygb, whereas binding of cyanide results in a 1.5-fold decrease in affinity of Cygb for the hydrophobic probe (Kd=76 μM). Molecular docking studies of 1,8-ANS with Cygb were performed and the data suggest that 1,8-ANS binds to the extended termini in Cygb.

Protein Folding & Stability II

2891-Pos Board B46
Effects of Sugars on the Thermal Stability of a Protein
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It is experimentally known that the heat-denaturation temperature of a protein is raised by sugar addition [1]. In earlier work [2], we proposed a measure of the thermal stability of a protein, which is defined as the solvent-entropy gain at 298 K upon protein folding ΔS normalized by the number of residues. ΔS was calculated using a hybrid method of the angle-dependent integral equation theory combined with the mulipolar water model and the morphometric approach. Here we show that ΔS remains effective even when the model water is replaced by the hard-sphere solvent whose number density and molecular diameter are set at those of water. We then investigate the effects of sugar addition on the thermal stability by considering water-sugar solution modeled as a binary mixture of hard spheres. The thermal stability is determined by a complex interplay of the molecular size of the sugar D and the total packing fraction of the solution η. D is estimated from the volume per molecule in the sugar crystal, and η is calculated using the experimental data of the solution density. A finding is that the protein is more stabilized as the sucrose or glucose concentration becomes higher and the stabilization effect is stronger for sucrose than for glucose. This is in accord with the experimental observations [1]. We analyze the contributions from the protein-solvent pair and many-body correlations to enhancement of the thermal stability. The contribution from the many-body correlation becomes stronger as the degree of thermal-stability enhancement increases, suggesting that the solvent crowding is made more serious by sugar addition, leading to an increase in the solvent-entropy gain upon folding.
[1] J. F. Back, Biochemistry 18 (1979) 5191.
[2] K. Oda *et al*., J. Chem. Phys. 134 (2011) 025101.

2892-Pos Board B47
New Powerful Protein Denaturant
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Traditional denaturants such as urea and guanidinium hydrochloride effectively unfold a variety of proteins in an ''all-or-none'' fashion. However their high working concentration in combination with the strong absorption below 210 nm make it impossible to measure high quality circular dichroism spectra, which are commonly used to detect changes in protein secondary structure. Detergents on the other hand destabilize native protein conformation at the extremely low concentration of several millimolar and are UV transparent, but they do not denature proteins as effectively as guanidinium or urea. In this work we studied the denaturation properties of the dodecylguanidine acetate which can be considered as a chemical combination of detergent and guanidinium. We have shown that dodecylguanidine acetate unfolds the protein at the millimolar concentration and is transparent enough to measure full range circular dichroism spectra. Our results also suggest that dodecylguanidine acetate allows to fine-tune the degree of protein unfolding unlike traditional ''all-or-none'' denaturants.

2893-Pos Board B48
The Influence of pH and Buffers on the Thermal Stability and Activity of RecA
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RecA is a DNA repair protein found in Escherichia coli, with homologues found in mammalian species. RecA is a DNA dependent ATPase that hydrolyzes ATP in the presence of single or double-stranded DNA. RecA has pH dependent affinities for ssDNA and dsDNA binding and ATP hydrolysis. Previous studies in our laboratory have shown that a variety of salts, substrates and pH conditions alter RecA structure and stability. In this study, three buffers were used to study the thermal unfolding and ATPase activity of RecA. RecA unfolding in HEPES, MES, and potassium phosphate buffers is compared to unfolding in Tris buffer at a variety of pH levels (6.5, 7.0, 7.5, 8.0, 8.5). Circular Dichroism was used to follow the unfolding transitions and to determine the melting temperature of RecA at each given pH in each of the various buffers. Activity assays were conducted for each of the solution conditions used for the CD studies in order to study how buffer composition and pH influences RecA activity. Differences in melting temperatures between buffers at a given pH suggest that the buffer itself may alter the aggregation state and stability of RecA.